FEBS 29214 FEBS Letters 579 (2005) 571–576

Minireview

The aggravating role of the ubiquitin–proteasome system in neurodegeneration

Helen C Ardley*, Chao-Chun Hung, Philip A Robinson

Molecular Medicine Unit, University of Leeds, St. James's University Hospital, Leeds LS9 7TF, UK

Received 13 December 2004; revised 14 December 2004; accepted 20 December 2004

Available online 11 January 2005

Edited by Sandro Sonnino

Abstract Association of protein inclusions or aggregates within brain tissues of patients with neurodegenerative disorders has been widely reported. These inclusions are commonly characterised both by the presence of ubiquitylated proteins and the sequestration of components of the ubiquitin–proteasome system (UPS). Such observations have led to the proposition that the UPS has a direct role in their formation. Indeed, the presence of ubiquitylated proteins and UPS components in inclusions may reflect unsuccessful attempts by the UPS to remove aggregating proteins. Whether the physical presence of inclusions causes cell death or, conversely, whether they are non-toxic and their presence reflects a cellular protective mechanism remains highly controversial.

Keywords: Ubiquitin; Neurodegeneration; Inclusion; Aggresome; Ubiquitin–proteasome system

1. Introduction

Neurodegenerative disorders, including Alzheimer's (AD) and Parkinson's (PD) diseases, are characterised by a selective loss of neurons in specific, but different, regions of the brain. The result is often a disruption to motor, sensory or cognitive systems, resulting in severe disability of the patient.

The pathological characteristic of many neurodegenerative diseases is the presence of distinctive ubiquitin-positive, intra- or extracellular, inclusion bodies in affected regions of the brain (Table 1). In general, these inclusions consist of insoluble, unfolded, ubiquitylated polypeptides that fail to be targeted and degraded by the 26S proteasome [1,2]. Their apparent stability may, in part, be due to decreased levels of 26S proteasomal activity that is associated with increasing age [3]. Given the pathological presentation of these conditions, it has not been altogether a major surprise to find that proteins associated with the UPS are now known to play either a direct or indirect role in familial forms of neurodegenerative disease and, in particular, PD.

2. The Ubiquitin–proteasome system

UPS-mediated post-translational modification and degradation of proteins is essential for most cellular processes such as cell cycling, DNA repair, cell signalling, gene transcription and apoptosis. Historically, it was recognised that the UPS is the major route by which proteins are selected for temporal and spatial degradation in eukaryotic cells [for recent reviews, see 4,5].

The covalent attachment of the seventy-six amino acid residue protein, ubiquitin, to an ε-amino group of a lysine residue in a target protein involves an energy-dependent, four-step pathway (Fig. 1). The UPS cascade requires transfer of ubiquitin from an ubiquitin-activating enzyme (E1) to an ubiquitin-conjugating enzyme (E2) and then to the target substrate protein facilitated by an ubiquitin-protein ligase (E3). Multiple rounds of ubiquitylation (generally through covalent attachment to ubiquitin Lys48) may occur, leading to the generation of polyubiquitin chains. Polyubiquitylated substrates are then selected for degradation by the 26S proteasome. At least four ubiquitin molecules must be attached to activate proteasomal mediated degradation. During proteasomal proteolysis ubiquitin is removed and recycled, and the target protein broken down into small peptides. These latter molecules can be hydrolysed further by other cellular peptidases or processed for MHC class 1 antigen presentation [6].

Until recently, the requirement for the addition of multiple ubiquitin molecules had remained somewhat of a mystery. It may provide a cellular proof-reading mechanism to protect certain proteins from degradation as a large number of de-ubiquitylating enzymes (DUBs) are also found within cells [7]. For such substrates, DUBs remove ubiquitin moieties before a sufficiently long ubiquitin chain has been synthesised to activate proteasomal destruction. Monoubiquitylation of proteins may also occur. This process appears to regulate endocytosis of cell surface receptors, DNA repair mechanisms, histone methylation and transcription regulation [8].

Several ubiquitin-like (Ubl) proteins such as SUMO and NEDD8 also covalently tag proteins [9,10]. However, in general, only single molecules are attached. Each requires its own unique combination of E1, E2 and E3. The addition of these tags does not target proteins for degradation, but has roles in activation of gene transcription, maintenance of multiprotein complex structures, protein localisation and protein stability.

3. Inclusion formation and the nature of aggresomes

The key constituents of the inclusions associated with neurodegenerative disorders are mis-folded proteins (Fig. 2). The

*Corresponding author.
E-mail address: h.c.ardley@leeds.ac.uk (H.C Ardley).

0014-5793/$30.00
doi:10.1016/j.febslet.2004.12.058

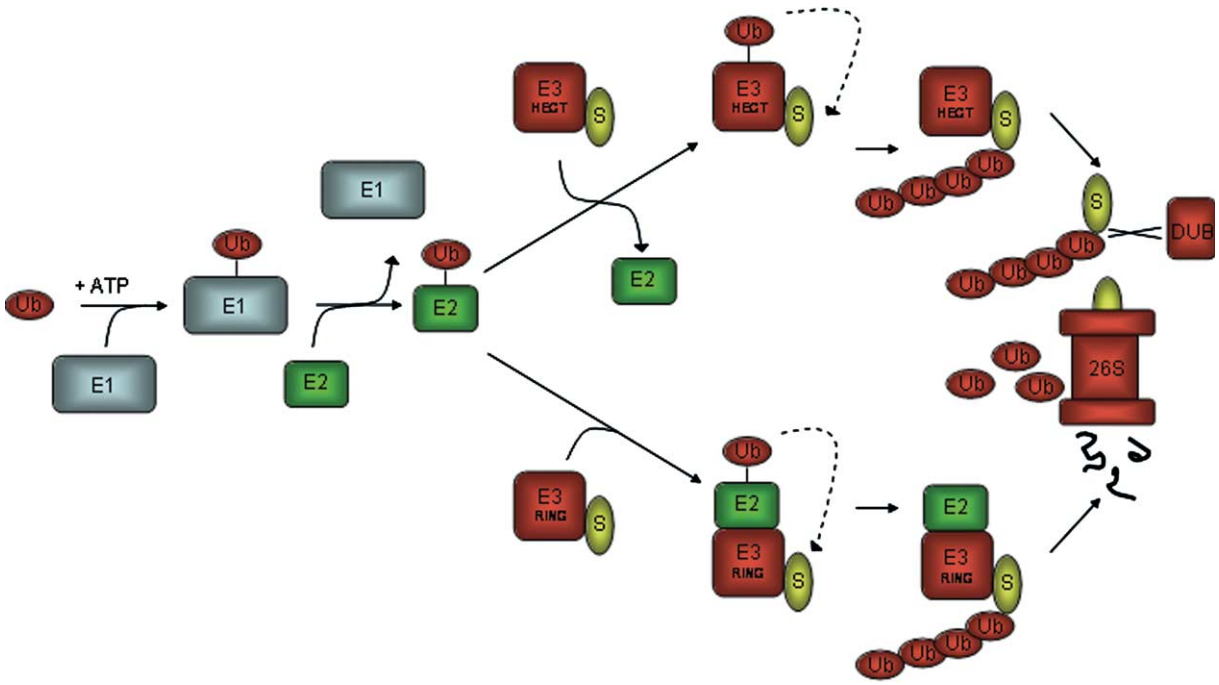

Fig. 1. Protein mediated degradation via the ubiquitin–proteasome pathway. Ubiquitylation occurs in a step-wise manner. An energy dependent thioester bond forms between the *C*-terminal glycine residue of ubiquitin (Ub) and a thiol group located within an ubiquitin-activating enzyme (E1). Ubiquitin is then transferred to an ubiquitin-conjugating enzyme (E2) via transthiolation. Ubiquitin-protein ligases (E3s) then facilitate ubiquitylation of the substrate protein (S). Several classes of E3s exist, most are either RING or HECT type E3s. HECT E3s transfer ubiquitin directly to the substrate, whereas RING E3s do not directly catalyse the target protein and may require the presence of additional components, including the E2, for ubiquitylation to proceed. Multiple ubiquitin molecules may then be covalently attached to one of several lysine residues found within a pre-attached ubiquitin to form a polyubiquitylated protein. Chains of at least four ubiquitin molecules are required for recognition by the 26S proteasome. Prior to ATP dependent proteasomal degradation, ubiquitins are removed from the substrate by one of many DUBs found within cells and the ubiquitin recycled for further targeted degradation. UPS components which can be mutated/dysfunctional in neurodegeneration are shown in red.

major causes of protein mis-folding and subsequent loss of function are mis-sense mutations, modifications or post-translational damage of proteins, or expansion of amino acid repeats as is observed in polyglutamine (polyQ) disorders such as Huntington's disease (HD).

The presence of these protein aggregates is somewhat puzzling given that the chaperone system and the UPS are designed to prevent such events occurring. Indeed, these inclusions are commonly associated with ubiquitin staining, indicating that cellular protective mechanisms attempt, yet somehow fail, to remove these abnormal proteins. Minor changes to the efficiency of the balance between synthesis and degradation may have disastrous long-term consequences for cells. These problems would be particularly acute in terminally differentiated non-renewing cell populations such as neurons.

Table 1
Neurodegenerative disorders associated with ubiquitylated inclusions

Neurodegenerative disease	Type of ubiquitylated inclusion
Alzheimer's	Amyloid plaques and neurofibrillary tangles
Parkinson's	Lewy bodies
Amyotrophic Lacteral sclerosis	Hydraline and skein-like
Huntington's and other PolyQ	Ubiquitylated inclusions
Pick's	Pick's bodies
Lafora	Starch-like polyglucosans (Lafora bodies)

Inclusions are routinely characterised by the presence of one major 'core' protein, or peptide, such as α-synuclein (the major constituent of Lewy bodies in PD) or β-amyloid peptide (Aβ, associated with the plaques of AD). It is assumed that these proteins provide the seed of an inclusion. However, inclusions are also characterised by a myriad of other proteins present at lower abundance, which are thought to be sequestered into inclusions as they form. These latter components often include chaperones [11,12], UPS components [1,13] and cytoskeletal elements [14,15]. In addition to seeding inclusion formation, mutant (but not wild-type) α-Synuclein and Aβ are capable of inhibiting proteasome activity, further exacerbating the problem [16,17].

In vitro cell culture systems are able to replicate many properties of neuronal inclusion formation. Using such systems, overexpression of a number of neurodegenerative disease-associated proteins, including Presenilin 1, Parkin and Huntingtin (associated with AD, PD and HD, respectively) leads to inclusion formation in the presence of proteasome inhibitors [18–21]. Moreover, many disease-associated mutant proteins readily form aggregates without the need for proteasome inhibition [20,21].

These inclusions have been characterised as 'aggresomes' [18,22]. It is postulated that aggresome formation is a protective cellular response to overloading of the proteasome [18]. They commonly contain chaperones, UPS components, centrosomal material and cytoskeletal proteins [18–22]. Most aggresomes are delivered in a microtubule-dependent manner to the

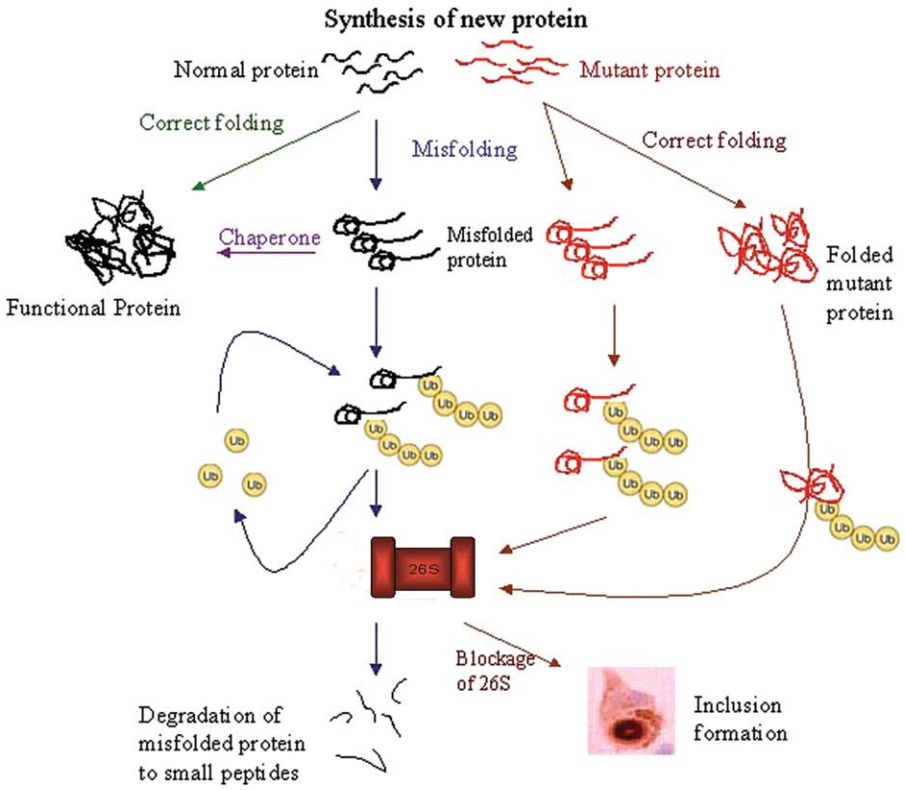

Fig. 2. Potential routes for the formation of inclusion bodies. Proteins which are not degraded by proteolysis may aggregate to form inclusion bodies. Under normal cellular conditions, functional proteins are generated through the correcting folding or refolding of proteins by molecular chaperones. The formation of aggregates may be prevented by degradation by the proteasome. Blockage of proteasomes by mis-folded protein may cause protein accumulation and aggregation that leads to the formation of inclusion bodies.

microtubule organising centre, where they become surrounded by a vimentin 'cage' [18,22]. McNaught and colleagues [23] recently proposed that Lewy bodies represent a specialised aggresome-related inclusion specific to dopaminergic neurons.

4. Neurodegenerative diseases and the malfunction of the UPS

Probably, the strongest evidence for a significant role of the UPS in these debilitating neurodegenerative diseases has come from genetic studies. Abnormal expression of these mutant proteins in combination with an age-related decline in proteasome activity provides a molecular explanation for the age-related nature of these disorders [3].

Ubiquitin itself does not escape aberrant properties as our cellular functions grow old. Molecular-misreading occurs when one or more base pairs are lost during the transcriptional process. In some AD patients, novel frameshift mutations in the ubiquitin B gene lead to translation of novel but nonsense peptide sequences creating 'ubiquitin + 1' [24,25]. Ubiquitin + 1 can accept additional ubiquitin molecules but cannot donate itself to an expanding polyubiquitin chain as it lacks its *C*-terminal glycine residue. This mutant form of ubiquitin blocks proteasomal degradation [24] and causes neuronal cell death [25].

To date, the E3 family appears to be the major group of components of the UPS to be affected in neurodegenerative disease (Fig. 1) [for recent review, see 26]. This is perhaps not surprising given that relatively few E1s and E2s exist in mammals. By contrast, there are potentially hundreds of E3s to be found within the human genome, the majority of which remain uncharacterised. Such a variety of E3s allows precise targeting of each (post-translationally modified) substrate at a given time or localisation within each cell in response to many different stimuli.

Multiple E3s may be involved in a single disease. Conversely, one E3 may play a part in more than one disease. For example, Parkin plays a significant role in PD, but may also provide a protective role in polyQ disorders [27]. Similarly, Dorfin is required to remove mutant SOD1 in the motor neuron disorder, Amyotrophic Lacteral Sclerosis, but may also be involved in synphilin-1 clearance in PD [28]. The pathological significance of such interplay between E3s as causative events in disease remains to be elucidated.

The most widely studied DUB associated with neurodegeneration is UCH-L1 (PGP 9.5), a protein highly abundant in neuronal cells [29]. Surprisingly, it was recently discovered that UCH-L1 also possesses a dimerisation dependent E3 activity towards α-synuclein in vitro [30]. UCH-L1 has been localised to inclusions associated with PD, AD and the Rosenthal fibres associated with cerebellar astrocytomas [31]. Moreover, different isoforms may play pathological or protective roles in AD, PD and HD [32–34]. The *gad* mouse is unable to express the murine orthologue of UCH-L1. Pathologically, it displays neuronal degeneration with progressive accumulation of Aβ and ubiquitin-positive inclusions along sensory and motor neurons [35]. These findings provide additional evidence that altered function of the UPS directly causes neurodegeneration, the *gad* mouse thus represents an ideal model in which to study the role of the UPS in such disorders.

As already discussed, as we grow older, the level of proteasomal activity in cells declines [3]. The consequence is likely to be less efficient clearance of proteins by the UPS. Presence of mutant and/or aggregated proteins is likely to compound these adverse effects by physically blocking entrance to the proteasome, further reducing its activity. As well as general blocking of the proteasome, some neurodegenerative disease associated proteins such as Ataxin-7, a polyQ containing protein associated with spinocerebellar ataxia type 7, can interact with 19S regulatory proteasomal subunits [36]. The 19S regulatory complex is responsible for recognition and binding of substrates prior to their entry into the 20S catalytic core of the proteasome. Expanded polyQs of Ataxin-7 bind poorly with the S4 subunit of the 19S regulatory complex suggesting that this may cause further interference of normal proteasomal functions.

4.1. Parkinson's disease

Of all the neurodegenerative diseases, PD is most closely associated with aberrant protein processing via the UPS. Indeed, of the known proteins associated with hereditary forms of PD, Parkin and UCH-L1 are components of the UPS, whereas modified and/or mutant α-Synuclein and DJ-1 are degraded by the system [37–41].

Hereditary mutations within the gene encoding Parkin cause loss of E3 activity without formation of the Lewy bodies [42]. A number of Parkin substrates have been identified including α-Synuclein [40] and/or a glycosylated form of α-Synuclein, termed α Sp22 [39]. Nonetheless, the significance of Parkin targeted ubiquitylation of these proteins with respect to PD remains largely unclear, since *Parkin*$^{-/-}$ mice display normal levels of a number of its known substrates [43]. Such observations may in part be due to a redundancy in substrate targeting by E3s such as CHIP, Dorfin and Siah-1, which are capable of targeting the removal of some of Parkin's substrates [28,44,45].

Mutations in the DUB UCH-L1, which drastically reduce its enzymatic activity, cause autosomal dominant PD in a German kindred [38]. Although it remains to be established whether these patients have Lewy bodies, the disease causing mutation has a high propensity to form aggresomes when overexpressed in vitro relative to the wild-type isoform [46].

5. Neurodegenerative inclusions: toxic or protective?

Over the years, there has been much debate regarding the presence of inclusions in neuronal cells of neurodegenerative disease patients. Do they reflect the results of a protective response mechanism to some yet to be identified adverse environmental challenge? Are they, or their precursors, toxic or non-toxic? In PD, inclusions are only found in the surviving neurons of the diseased patients. Did the dopaminergic neurons that have died once contain toxic inclusions? Or were non-surviving cells unable to form inclusions to protect themselves from toxic entities resulting in their death?

The results of recent studies indicate that the 'Toxic or Protective?' argument is probably a simplistic view of what is happening inside patients' affected cells. Recent studies have indicated that prolonged low-level proteasome inhibition causes buildup of aggregated protein species within neuronal cells in vitro [47]. In addition, exposure to proteasome inhibitors in rats caused progressive parkinsonism with dopaminergic cell loss [48]. Furthermore, studies of aggregate prone proteins such as Aβ indicate that mis-folded intermediates, or protofibrils, generated during the production of amyloid fibrils (which may subsequently form AD plaques) are highly toxic, possibly more so than the fibril itself [49,50]. Indeed, there is increasing evidence that inclusions and aggresomes are cytoprotective [e.g. 51,52] and may activate autophagy in order to remove the mutant protein [53,54].

In summary, it appears likely that the protofibrillar form of many neurodegenerative disease associated proteins may be guilty of causing rapid cell death (hence, inherited forms of these disorders tend to have an earlier onset than the sporadic forms). Yet if the protofibrils can be made to aggregate rapidly into inclusions, the cell becomes somewhat protected from the damage these toxic entities cause and may even be able to clear the aggregate via the proteasome or autophagy. In the longer term, however, aggregation may exceed clearance of proteins and cause cellular death.

6. Concluding remarks

The UPS is by no means the only culprit in the causation of neurodegeneration. Although their precise roles in disease remain unclear, ubiquitin related proteins such as SUMO and the ubiquitin binding protein p62 are common constituents of the pathological inclusions associated with neurodegenerative diseases [55,56].

Furthermore, similar to the proteasome, the efficiency of the chaperone system is also thought to deteriorate with age [57]. Mitochondrial dysfunction and excessive production of reactive oxygen species (ROS) are also known causes of neurodegeneration [58,59]. The cumulative effect of inefficient chaperone and UPS activity, and a malfunctioning mitochondrial respiratory chain (leading to lower ATP levels and increases in ROS and oxidative damage) produces a vicious cycle. Understanding the interplay between these systems, and where they may overlap in different disorders, is a complex and overwhelming task. However, it may be the only way we can truly find efficient, disease specific, curative and preventative medicines for these debilitating conditions.

Acknowledgements: H.C.A. is a recipient of a 'Research into Ageing' Fellowship award. Work in the Authors' laboratory is also supported by The Parkinson's Disease Society (UK).

References

[1] Lowe, J., Blanchard, A., Morrell, K., Lennox, G., Reynolds, L., Billett, M., Landon, M. and Mayer, R.J. (1988) Ubiquitin is a common factor in intermediate filament inclusion bodies of diverse type in man, including those of Parkinson's disease, Pick's disease, and Alzheimer's disease, as well as Rosenthal fibres in cerebellar astrocytomas, cytoplasmic bodies in muscle, and mallory bodies in alcoholic liver disease. J. Pathol. 155, 9–15.
[2] Sherman, M.Y. and Goldberg, A.L. (2001) Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron 29, 15–32.
[3] Goto, S., Takahashi, R., Kumiyama, A., Radak, Z., Hayashi, T., Takenouchi, M. and Abe R (2001) Implications of protein degradation in aging. Ann. N. Y. Acad. Sci. 928, 54–64.
[4] Pickart, C.M. (2004) Back to the future with ubiquitin. Cell 116, 181–190.

[5] Muratani, M. and Tansey, W.P. (2003) How the ubiquitin–proteasome system controls transcription. Nat. Rev. Mol. Cell Biol. 4, 192–201.
[6] Kloetzel, P.-M. (2001) Antigen processing by the proteasome. Nat. Rev. Mol. Cell Biol. 2, 179–187.
[7] Baek (2003) Conjugation and deconjugation of ubiquitin regulating the destiny of proteins. Exp. Mol. Med. 35, 1–7.
[8] Hicke, L. (2001) Protein regulation by monoubiquitin. Nat. Rev. Mol. Cell Biol. 2, 195–201.
[9] Muller, S., Hoege, C., Pyrowolakis, G. and Jentsch, S. (2001) SUMO, ubiquitin's mysterious cousin. Nat. Rev. Mol. Cell Biol. 2, 202–210.
[10] Schwartz, D.C. and Hochstrasser, M. (2003) A superfamily of protein tags: ubiquitin, SUMO and related modifiers. Trends Biochem. Sci. 28, 321–328.
[11] Renkawek, K., Stege, G.J and Bosman, G.J. (1999) Dementia, gliosis and expression of the small heat shock proteins hsp27 and alpha B-crystallin in Parkinson's disease. Neuroreport 10, 2273–2276.
[12] Lowe, J., McDermott, H., Pike, I., Spendlove, I., Landon, M. and Mayer, R.J. (1992) Alpha B crystallin expression in non-lenticular tissues and selective presence in ubiquitinated inclusion bodies in human disease. J. Pathol. 166, 61–68.
[13] Fergusson, J., Landon, M., Lowe, J., Dawson, S.P., Layfield, R., Hanger, D.P. and Mayer, R.J. (1996) Pathological lesions of Alzheimer's disease and dementia with Lewy bodies brains exhibit immunoreactivity to an ATPase that is a regulatory subunit of the 26S proteasome. Neurosci. Lett. 219, 167–170.
[14] Seitelberger, F., Lassmann, H. and Bancher, C. (1991) Cytoskeleton pathology in Alzheimer's disease and related disorders. J. Neural. Transm. Suppl. 33, 27–33.
[15] Nakazato, Y., Sasaki, A., Hirato, J. and Ishida, Y. (1984) Immunohistochemical localization of neurofilament protein in neuronal degenerations. Acta Neuropathol. 64, 30–36.
[16] Gregori, L., Fuchs, C., Figueiredo-Pereira, M.E., Van Nostrand, W.E. and Goldgaber, D. (1995) Amyloid beta-protein inhibits ubiquitin-dependent protein degradation in vitro. J. Biol. Chem. 270, 19702–19708.
[17] Tanaka, Y., Engelender, S., Igarashi, S., Rao, R.K., Wanner, T., Tanzi, R.E., Sawa, A., Dawson, V.L., Dawson, T.M. and Ross, C.A. (2001) Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. Hum. Mol. Genet. 10, 919–926.
[18] Johnston, J.A., Ward, C.L. and Kopito, R.R. (1998) Aggresomes: a cellular response to misfolded proteins. J. Cell Biol. 143, 1883–1898.
[19] Junn, E., Lee, S.S., Suhr, U.T. and Mouradian, M.M. (2002) Parkin accumulation in aggresomes due to proteasome impairment. J. Biol. Chem. 277, 47870–47877.
[20] Ardley, H.C., Scott, G.B., Rose, S.A., Tan, N.G.S., Markham, A.F. and Robinson, P.A. (2003) Inhibition of proteasomal activity causes inclusion formation in neuronal and non-neuronal cells overexpressing Parkin. Mol. Biol. Cell 14, 4541–4556.
[21] Waelter, S., Boeddrich, A., Lurz, R., Scherzinger, E., Lueder, G., Lehrach, H. and Wanker, E.E. (2001) Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. Mol. Biol. Cell 12, 1393–1407.
[22] Garcia-Mata, R., Gao, Y.-S. and Sztul, E. (2002) Hassles with taking out the garbage: aggravating aggresomes. Traffic 3, 388–396.
[23] McNaught, K.St.P., Shashidharan, P., Perl, D.P., Jenner, P. and Olanow, C.W. (2002) Aggresome-related biogenesis of Lewy bodies. Eur. J. Neurosci. 16, 2136–2148.
[24] Lindsten, K., de Vrij, F.M., Verhoef, L.G., Fischer, D.F., van Leeuwen, F.W., Hol, E.M., Masucci, M.G. and Dantuma, N.P. (2002) Mutant ubiquitin found in neurodegenerative disorders is a ubiquitin fusion degradation substrate that blocks proteasomal degradation. J. Cell Biol. 157, 417–427.
[25] Hope, A.D., de Silva, R., Fischer, D.F., Hol, E.M., van Leeuwen, F.W. and Lees, A.J. (2003) Alzheimer's associated variant ubiquitin causes inhibition of the 26S proteasome and chaperone expression. J. Neurochem. 86, 394–404.
[26] Ardley, H.C. and Robinson, P.A. (2004) The role of ubiquitin protein ligases in neurodegenerative disease. Neurodegenerative Dis. 1, 71–87.
[27] Tsai, Y.C., Fishman, P.S., Thakor, N.V. and Oyler, G.A. (2003) Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. J. Biol. Chem. 278, 22044–22055.
[28] Ito, T., Niwa, J.-i., Hishikawa, N., Ishigaki, S., Doyu, M. and Sobue, G. (2003) Dorfin localizes to Lewy bodies and ubiquitylates synphilin-1. J. Biol. Chem. 278, 29106–29114.
[29] Wilkinson, K.D., Lee, K.M., Deshpande, S., Duerksen-Hughes, P., Boss, J.M. and Pohl, J. (1989) The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. Science 246, 670–673.
[30] Liu, Y., Fallon, L., Lashuel, H.A., Liu, Z. and Lansbury Jr., P.T. (2002) The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. Cell 111, 209–218.
[31] Lowe, J., McDermontt, H., Landon, M., Mayer, R.J. and Wilkinson, K.D. (1990) Ubiquitin carboxyl-terminal hydrolase (PGP 9.5) is selectively present in ubiquitinated inclusion bodies characteristic of human neurodegenerative diseases. J. Pathol. 161, 153–160.
[32] Maraganore, D.M., de Andrade, M., Lesnick, T.G., Farrer, M.J., Bower, J.H., Hardy, J.A. and Rocca, W.A. (1999) Case-control study of the ubiquitin carboxy-terminal hydrolase L1 gene in Parkinson's disease. Neurology 53, 1858–1860.
[33] Naze, P., Vuillaume, I., Destee, A., Pasquier, F. and Sabloniere, B. (2002) Mutation analysis and association studies of the ubiquitin carboxy-terminal hydrolase L1 gene in Huntington's disease. Neurosci. Lett. 328, 1–4.
[34] Choi, J., Levey, A.I., Weintraub, S.T., Rees, H.D., Gearing, M., Chin, L.-S. and Li, L. (2004) Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. J. Biol. Chem. 279, 13256–13264.
[35] Saigoh, K., Wang, Y.-L., Suh, J.-G., Yamanishi, T., Sakai, Y., Kiyosawa, H., Harada, T., Ichihara, N., Wakana, S., Kikuchi, T. and Wada, K. (1999) Intragenic deletion in the gene encoding ubiquitin carboxy-terminal hydrolase in gad mice. Nat. Genet. 23, 47–51.
[36] Matilla, A., Gorbea, C., Einum, D.D., Townsend, J., Michalik, A., van Broeckhoven, C., Jensen, C.C., Murphy, K.J., Ptacek, L.J. and Fu, Y.-C. (2001) Association of ataxin-7 with the proteasome subunit S4 of the 19S regulatory complex. Hum. Mol. Genet. 10, 2821–2831.
[37] Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. and Shimizu, N. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392, 605–608.
[38] Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M.J., Jonnalagada, S., Chernova, T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P.J., Wilkinson, K.D. and Polymeropoulos, M.H. (1998) The ubiquitin pathway in Parkinson's disease. Nature 395, 451–452.
[39] Shimura, H., Schlossmacher, M.G., Hattori, N., Frosch, M.P., Trockenbacher, A., Schneider, R., Mizuno, Y., Kosik, K.S. and Selkoe, D.J. (2001) Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. Science 293, 263–269.
[40] Choi, P., Golts, N., Snyder, H., Chong, M., Petrucelli, L., Hardy, J., Sparkman, D., Cochran, E., Lee, J.M. and Wolozin, B. (2001) Co-association of parkin and alpha-synuclein. Neuroreport 12, 2839–2843.
[41] Miller, D.W., Ahmad, R., Hague, S., Baptista, M.J., Canet-Aviles, R., McLendon, C., Carter, D.M., Zhu, P.P., Stadler, J., Chandran, J., Klinefelter, G.R., Blackstone, C. and Cookson, M.R. (2003) L166P mutant DJ-1, causative for recessive Parkinson's disease, is degraded through the ubiquitin–proteasome system. J. Biol. Chem. 278, 36588–36595.
[42] Mori, H., Kondo, T., Yokochi, M., Matsumine, H., Nakagawa-Hattori, Y., Miyake, T., Suda, K. and Mizuno, Y. (1998) Pathologic and biochemical studies of juvenile parkinsonism linked to chromosome 6q. Neurology 51, 890–892.

[43] Goldberg, M.S., Fleming, S.M., Palacino, J.J., Cepeda, C., Lam, H.A., Bhatnagar, A., Meloni, E.G., Wu, N.P., Ackerson, L.C., Klapstein, G.J., Gajendiran, M., Roth, B.L., Chesselet, M.F., Maidment, N.T., Levine, M.S. and Shen, J. (2003) Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. J. Biol. Chem. 278, 43628–43635.
[44] Imai, Y., Soda, M., Hatakeyama, S., Akagi, T., Hashikawa, T., Nakayama, K-I. and Takahashi, R. (2002) CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. Mol. Cell 10, 55–67.
[45] Nagano, Y., Yamashita, H., Takahashi, T., Kishida, S., Nakamura, T., Iseki, E., Hattori, N., Mizuno, Y., Kikuchi, A. and Matsumoto, M. (2003) Siah-1 facilitates ubiquitination and degradation of synphilin-1. J. Biol. Chem. 278, 51504–51514.
[46] Ardley, H.C., Scott, G.B., Rose, S.A., Tan, N.G.S. and Robinson, P.A. (2004) UCH-L1 aggresome formation in response to proteasome impairment indicates a role in inclusion formation in Parkinson's disease. J. Neurochem. 90, 379–391.
[47] Ding, Q., Dimayuga, E., Martin, S., Bruce-Keller, A.J., Nukala, V., Cuervo, A.M. and Keller, J.N. (2003) Characterization of chronic low-level proteasome inhibition on neural homeostasis. J. Neurochem. 86, 489–497.
[48] McNaught, K.P., Perl, D.P., Brownell, A-L. and Olanow, C.W. (2004) Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. Ann. Neurol. 56, 149–162.
[49] Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C.M. and Stefani, M. (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 416, 507–511.
[50] Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J. and Selkoe, D.J. (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416, 535–539.
[51] Taylor, J.P., Tanaka, F., Robitschek, J., Sandoval, C.M., Taye, A., Markovic-Plese, S. and Fischbeck, K.H. (2003) Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. Hum. Mol. Genet. 12, 749–757.
[52] Tanaka, M., Kim, Y.M., Lee, G., Junn, E., Iwatsubo, T. and Mouradian, M.M. (2004) Aggresomes formed by alpha-synuclein and synphilin-1 are cytoprotective. J. Biol. Chem. 279, 4625–4631.
[53] Ravikumar, B., Vacher, C., Berger, Z., Davies, J.E., Luo, S., Oroz, L.G., Scaravilli, F., Easton, D.F., Duden, R., O'Kane, C.J. and Rubinsztein, D.C. (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat. Genet. 36, 585–595.
[54] Keller, J.N., Dimayuga, E., Chen, Q., Thorpe, J., Gee, J. and Ding, Q. (2004) Autophagy, proteasomes, lipofuscin, and oxidative stress in the aging brain. Int. J. Biochem. Cell Biol. 36, 2376–2391.
[55] Kuusisto, E., Salminen, A. and Alafuzoff, I. (2001) Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. Neuroreport 12, 2085–2090.
[56] Terashima, T., Kawai, H., Fujitani, M., Maeda, K. and Yasuda, H. (2002) SUMO-1 co-localized with mutant atrophin-1 with expanded polyglutamines accelerates intranuclear aggregation and cell death. Neuroreport 13, 2359–2364.
[57] Soti, C. and Csermely, P. (2003) Aging and molecular chaperones. Exp. Gerontol. 38, 1037–1040.
[58] Zhang, Y., Dawson, V.L. and Dawson, T.M. (2000) Oxidative stress and genetics in the pathogenesis of Parkinson's disease. Neurobiol. Dis. 7, 240–250.
[59] Hashimoto, M., Rockenstein, E., Crews, L. and Masliah, E. (2003) Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases. Neuromol. Med. 4, 21–36.